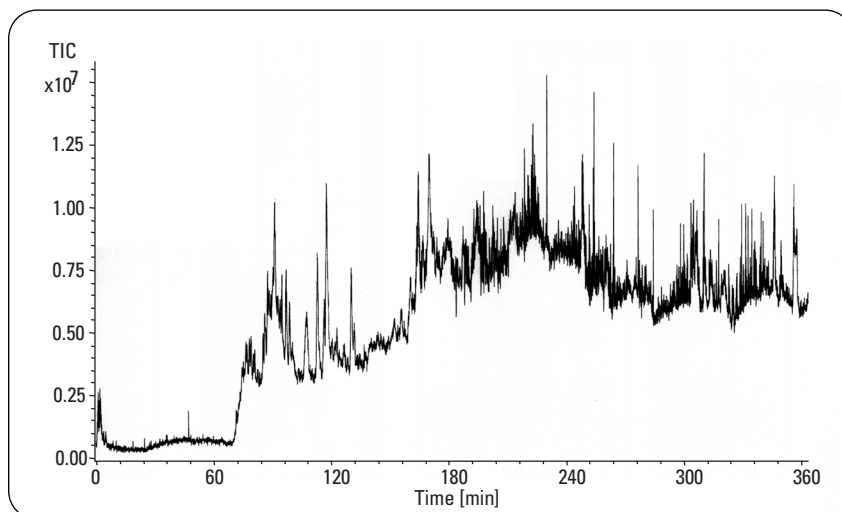


**Figure 1**  
**MS<sup>n</sup> spectra of ergotamine (A) and ergometrine (B)**

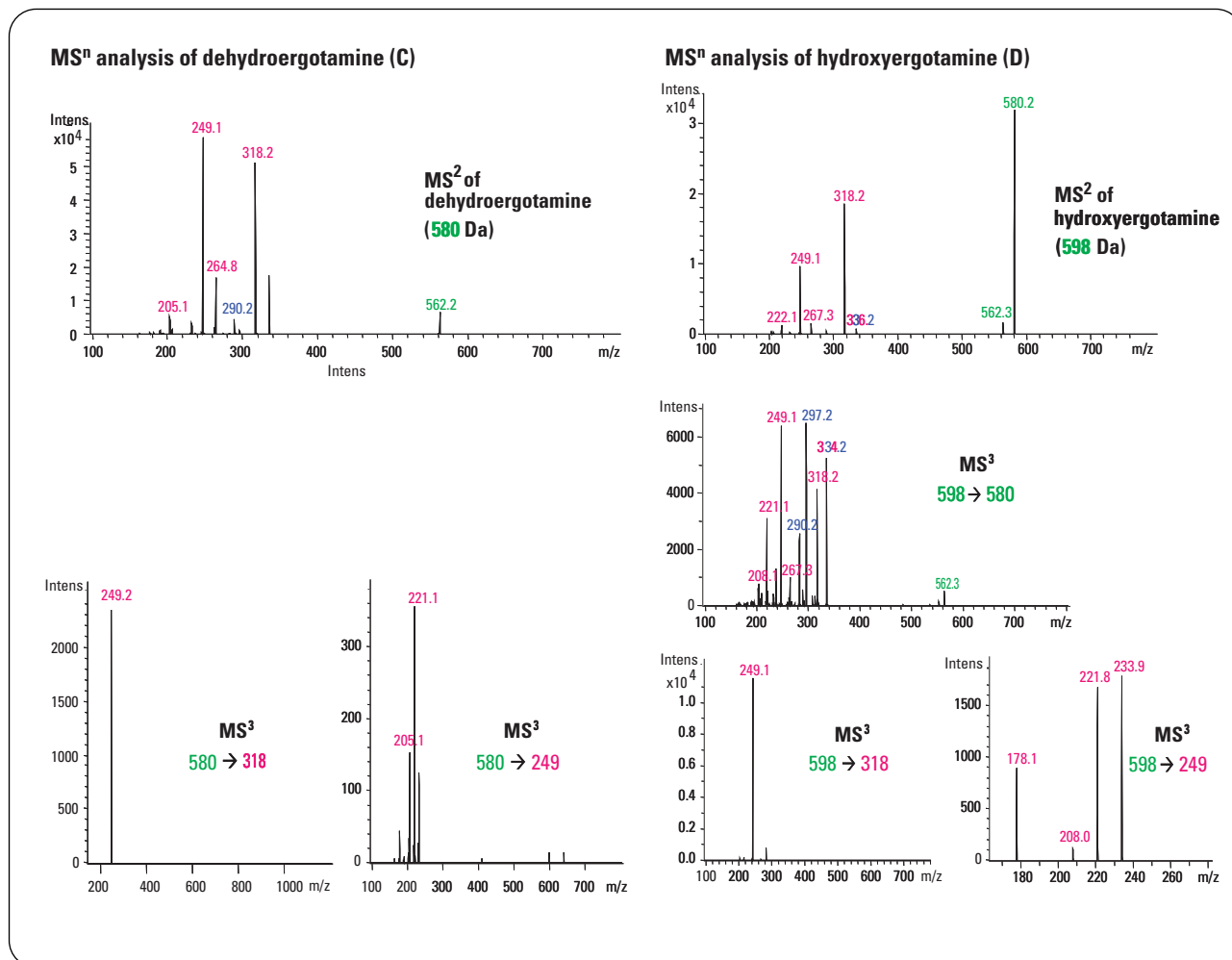
## Materials and methods

**Sample:** The crude extract of ergot fungus was purchased from Firma Dr. Hetterich (Fuerth, Germany), 0.22  $\mu$ m filtrated and directly injected.

**Analysis:** In a first analysis using the Agilent 1100 Series nano LC system directly coupled to an Agilent ion trap mass spectrometer, substances showing the lysergic acid structure (m/z 223 or m/z 208) (structures front page and figure 1) were identified by



**Figure 2**  
**Total ion chromatogram of the separation of the crude ergot extract**



**Figure 3**  
**MS<sup>n</sup> spectra of dehydroergotamine (C) and hydroxyergotamine (D)**

LC/MS/MS experiments. To confirm the structure of already known alkaloids LC/MS<sup>n</sup> experiments were carried out. To achieve thorough characterization of the unknown alkaloids they were purified using an Agilent 1100 Series purification system in mass-based fraction collection mode. The purified alkaloids were then structurally characterized with flow injection MS<sup>n</sup> experiments thus allowing to optimize fragmentation parameters for each ion individually.

## Results and discussion

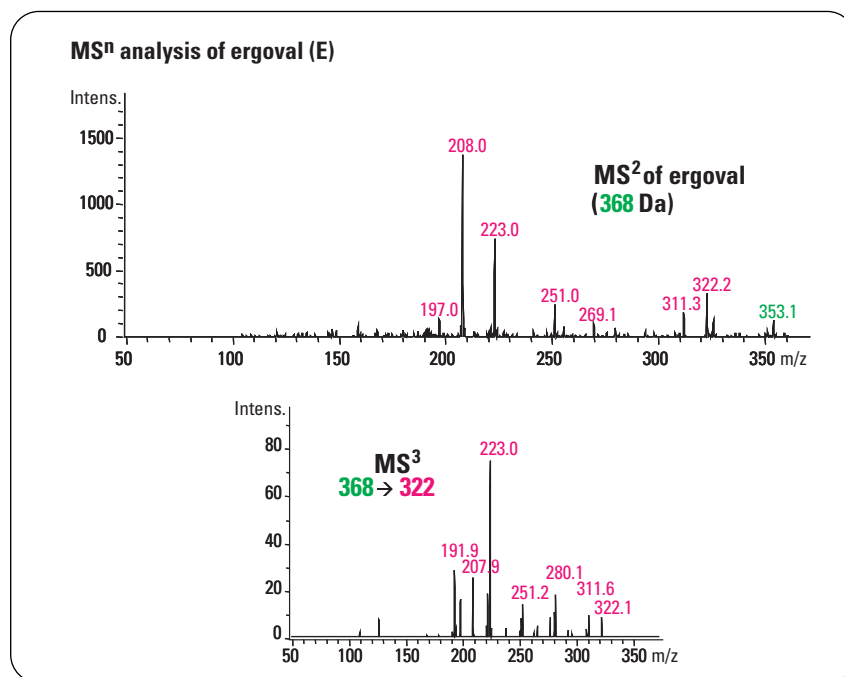
The analytical chromatogram of the crude ergot extract is shown in figure 2. It clearly demonstrates the large amount of different substances that are present in the sample. Using the technique described above the two well known alkaloids ergotamine and ergometrine were identified (figure 1)<sup>2, 3, 4</sup>. Additionally, three so far unknowns were found. They were purified and structural investigations were done as described

before. The comparison of their MS<sup>n</sup> spectra to those of the known derivatives allowed to characterize the derivatives and led to the structural proposals shown in figures 3 and 4. Dehydroergotamine is an oxidized derivative of ergotamine whereas in hydroxyergotamine the amino acid alanine has been replaced by serine. In ergoval the lysergic acid is esterified with the amino acid valine. As this amino acid is also used in other alkaloids of the tricyclic peptide type 4 it may either

represent a pre-state or a reduction product of other forms, but these could not be found within the extract used. Thus it is more likely that the valine is the “final” product.

## Conclusion

We demonstrated that nano-LC-MS/MS screening allows to identify analytes possessing a certain structure out of a crude mixture. Their purification followed by mass-based fraction collection allows their structural characterization with MS<sup>n</sup>. Apart from two well-known ergot alkaloids three so far unknowns have been identified and structurally characterized by MS<sup>n</sup> experiments. Based on these spectra their structures could be proposed.



**Figure 4**  
MS<sup>n</sup> spectra of ergoval (E)

## References

1. “Methode zur Bestimmung von Mutterkornalkaloiden in Lebensmitteln“, Klug, C.; Baltes, W.; Krönert, W.; Weber, R., *Z Lebensm Unters Forsch*, 186, 108 – 113, **1988**.
2. “Analysis of ergot alkaloids in endophyte infected tall fescue by liquid chromatography electrospray ionisation mass spectrometry“, Shelby, R. A.; Olsovska, J.; Havlicek, V.; Flieger, M.; *J. Agric. Food Chem.*, 45, **1997**.

3. “Untersuchungen zur Massenspektrometrie von Mutterkornalkaloiden“, Voigt, D.; John, S.; Gröger, D., *Pharmazie*, 29, H. 10 – 11, 697 – 700, **1974**.
4. “Mass spectrometric amino acid structure determination in ergopeptides“, 4. Halada, P.; Sedmera, P.; Havlicek, V.; Jegorov, A.; Cvak, L.; Ryska, M., *Eur. Mass Spectrometry*, 4, 385 – 392, **1998**.

*Mark Stahl and Edgar Nägele are Application Chemists at Agilent Technologies GmbH, Waldbronn, Germany.*

[www.agilent.com/chem/1100](http://www.agilent.com/chem/1100)

The information in this publication is subject to change without notice.

Copyright © 2003 Agilent Technologies  
All Rights Reserved. Reproduction, adaptation or translation without prior written permission is prohibited, except as allowed under the copyright laws.

Published November 1, 2003  
Publication number 5989-0261EN



**Agilent Technologies**